EFSA NDA Panel (EFSA Panel on Dietetic Products, Nutrition and Allergies), 2015. Scientific Opinion on the substantiation of a health claim related to a combination of pomegranate pomace extract and greater galangal rhizome powder and an increase in the number of motile spermatozoa in semen pursuant to Article 13(5) of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to a combination of pomegranate pomace extract and greater galangal rhizome powder and an increase in the number of motile spermatozoa in semen pursuant to Article 13(5) of Regulation (EC) No 1924/2006[1]

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ABSTRACT

Following an application from Nerthus ApS, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Denmark, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to a combination of pomegranate pomace extract (standardised by its content of punicalagins) and greater galangal rhizome powder (standardised by its content of acetoxychavicol acetate) and an increase in the number of motile spermatozoa in semen. The Panel considers that the food is sufficiently characterised. An increase in the number of motile spermatozoa in semen is a beneficial physiological effect. In weighing the evidence, the Panel took into account that one human study showed an increase in the number of motile spermatozoa in semen when the combination of pomegranate pomace extract and greater galangal rhizome powder was consumed for three months, that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which the food could exert the claimed effect. The Panel concludes that a cause and effect relationship has not been established between the consumption of the combination of pomegranate pomace extract (standardised by its content of punicalagins) and greater galangal rhizome powder (standardised by its content of acetoxychavicol acetate) and an increase in the number of motile spermatozoa in semen.

KEY WORDS

pomegranate, greater galangal, semen, spermatozoa, health claims

[1] On request from the Competent Authority of Denmark following an application by Nerthus ApS, Question No EFSA-Q-2014-00566, adopted on 22 April 2015.

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[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Marina Heinonen, Ambroise Martin, Hildegard Przyrembel, Yolanda Sanz, Alfonso Siani, Anders Sjödin, Sean (J.J.) Strain, Inge Tetens, Hendrik Van Loveren, Hans Verhagen and Peter Willatts, for the preparatory work on this scientific opinion.

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SUMMARY

Following an application from Nerthus ApS, submitted for authorisation of a health claim pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Denmark, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver an opinion on the scientific substantiation of a health claim related to a combination of pomegranate pomace extract and greater galangal rhizome powder and an increase in the number of motile spermatozoa in semen.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.

The food that is the subject of the health claim is a combination of pomegranate (*Punica granatum* L.) pomace extract and greater galangal (*Alpinia galanga* (L.) Willd.) rhizome powder. The Panel considers that the food, a combination of pomegranate pomace extract (standardised by its content of punicalagins) and greater galangal rhizome powder (standardised by its content of acetoxychavicol acetate), is sufficiently characterised.

The claimed effect is "increases the number of motile spermatozoa in semen". The target population proposed by the applicant is "men from the normal population with a wish to father a child". Spermatozoa are constituents of normal semen and are needed for fertilisation of the female ova. The total number of spermatozoa per ejaculate as well as their properties, i.e. motility, viability and morphology, are key determinants of male fertility. Increasing the number of motile spermatozoa in semen may contribute to the fertility of men. The Panel considers that an increase in the number of motile spermatozoa in semen is a beneficial physiological effect.

The applicant provided one unpublished intervention study which assessed the effects of the food that is the subject of the health claim on the number of motile spermatozoa in semen (i.e. the claimed effect) *in vivo* in humans.

This double-blind, randomised, controlled, parallel trial was carried out in 70 Danish men who received pomegranate extract (1 000 mg per day) plus greater galangal powder (764 mg per day) or a placebo for three months. The primary outcome of the study was total motile sperm count (TMSC). Sperm morphology was assessed as a secondary outcome. For the statistical analysis, an unequal variance t-test was used. In a secondary analysis, adjustments were made in a linear regression model for age and body mass index (BMI), both dichotomised at the median. When the mean changes (i.e. end of the study versus baseline) in TMSC were compared, a statistically significant difference (+10.5 million; 95 % confidence interval (CI): 1.3–19.7, p = 0.026) was found between the pomegranate/greater galangal-group (+14.5 ± 21.3 million) and the control group (+4.0 ± 15.2 million). When the analysis was adjusted for age and BMI, the difference between the groups remained significant (+9.8 million; 95 % CI: 0.2–19.5; p = 0.047). No differences were found for the secondary outcome between the groups. The Panel notes that this study shows an effect of the food on an increase in the number of motile spermatozoa in semen.

With regards to the mechanism by which the food could exert the claimed effect, the applicant claimed that data from some human and animal studies suggest a complementary mode of action for each of the two major constituents of the food that is the subject of the claim: greater galangal would increase blood testosterone concentrations, whereas pomegranate would exert a "direct antioxidant effect" through ellagic acid and urolithins, and an "indirect antioxidant effect" by up-regulating serum paraoxonase, which would lead to lower oxidative stress and to the protection of sperm from oxidative damage.

The applicant provided 18 human studies, 20 animal studies and two *in vitro* studies in support of a mechanism by which the two major constituents of the food could exert the claimed effect.

Two human studies and three animal studies were submitted only as abstracts, which did not allow a full scientific evaluation by the Panel. Therefore, no conclusions can be drawn from these studies on the mechanism by which the food could exert the claimed effect.

The remaining 16 human studies were carried out with various preparations of pomegranate or isolated compounds thereof. The Panel notes that these studies did not assess whether or not the food could protect sperm from oxidative damage, or the extent to which the protection of sperm from oxidative damage could result in an increase in the number of motile spermatozoa in semen.

The 17 animal studies were performed in a variety of species (i.e. mice, rats, rabbits and roosters) and models (e.g. animals with chemically induced testicular and/or spermatozoal toxicity). Two animal studies investigated the effects of greater galangal extracts. The Panel notes that these studies do not provide evidence that greater galangal extracts induce an increase in plasma testosterone concentrations which would affect the number of motile spermatozoa in semen. The remaining 15 animal studies investigated the effects of various preparations of pomegranate. The Panel considers that these studies do not provide evidence for an effect of the pomegranate preparations used on the protection of sperm against oxidative damage or on the extent to which the protection of sperm against oxidative damage might increase the number of motile spermatozoa in semen. The Panel also notes that these studies do not provide evidence that changes in plasma testosterone concentrations would consistently affect the number of motile spermatozoa in semen.

The two *in vitro* studies were performed in human immortalised cell lines and investigated the redox properties of various urolithins and urolithin derivatives. The Panel notes that the capacity of foods to scavenge free radicals *in vitro* does not provide information about their potential to decrease oxidative damage to molecules *in vivo*.

The Panel considers that the human, animal and *in vitro* studies do not provide evidence for a mechanism by which a combination of pomegranate pomace extract and greater galangal rhizome powder could increase the number of motile spermatozoa in semen.

In weighing the evidence, the Panel took into account that one human study showed an increase in the number of motile spermatozoa in semen when the combination of pomegranate pomace extract and greater galangal rhizome powder was consumed for three months, that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which the food could exert the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of the combination of pomegranate pomace extract (standardised by its content of punicalagins) and greater galangal rhizome powder (standardised by its content of acetoxychavicol acetate) and an increase in the number of motile spermatozoa in semen.

TABLE OF CONTENTS

BACKGROUND

Regulation (EC) No 1924/2006[4] harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation, and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children's development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA

- The application was received on 04/08/2014.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence. The application included a request for the protection of proprietary data.
- On 16/09/2014, during the validation process of the application, EFSA sent a request to the applicant to provide missing information.
- On 22/09/2014, EFSA received the missing information as submitted by the applicant.
- The scientific evaluation procedure started on 22/10/2014.
- On 27/11/2014, the Working Group on Claims of the NDA Panel agreed on a list of questions for the applicant to provide additional information to accompany the application, and the scientific evaluation was suspended on 16/12/2014, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 26/12/2014, EFSA received the applicant's reply and the scientific evaluation was restarted, in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- During its meeting on 22/04/2015, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to a combination of pomegranate pomace extract and greater galangal rhizome powder and an increase in the number of motile spermatozoa in semen.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to: a combination of pomegranate pomace extract and greater galangal rhizome powder and an increase in the number of motile spermatozoa in semen.

[4] Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9–25.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation for the marketing of a combination of pomegranate pomace extract and greater galangal rhizome powder, a positive assessment of its safety, nor a decision on whether a combination of pomegranate pomace extract and greater galangal rhizome powder is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.

INFORMATION PROVIDED BY THE APPLICANT

Applicant's name and address

Nerthus ApS, Blaesenborgvej 9, DK-4320 Lejre, Denmark.

The application includes a request for the protection of proprietary data for one unpublished study (Fedder et al.), in accordance with Article 21 of Regulation (EC) No 1924/2006.

Food/constituent as stated by the applicant

According to the applicant, the food that is the subject of the health claim is a combination of an extract of the fruit pomace of pomegranate (*Punica granatum* L.), standardised to the content of punicalagins (min. 30 %), and freeze-dried powder of the rhizome of greater galangal (*Alpinia galanga* (L.) Willd.), standardised to the content of 1´*S*-1´-acetoxychavicol acetate (min. 4 %).

Health relationship as claimed by the applicant

According to the applicant, daily consumption of a combination of pomegranate pomace extract and greater galangal rhizome powder increases the number of motile spermatozoa in semen.

The applicant indicated that the exact mechanism by which the food might exert the claimed effect is not known. However, the applicant claims that some evidence suggests a testosterone-enhancing effect, primarily originating from greater galangal, and an antioxidant effect, primarily originating from pomegranate (comprising a "direct antioxidant effect", caused by ellagic acid and urolithins, and an "indirect antioxidant effect", caused by the up-regulation of serum paraoxonase).

Wording of the health claim as proposed by the applicant

The applicant has proposed the following wording for the health claim: "A combination of standardised pomegranate pomace extract and greater galangal rhizome powder increases the number of motile spermatozoa in semen".

Specific conditions of use as proposed by the applicant

The applicant has proposed a daily intake of 1 000 mg pomegranate pomace extract (containing at least 300 mg of punicalagins and at least 400 mg of punicalagins, punicalins, ellagic acid glycosides and ellagic acid) and 764 mg of greater galangal rhizome powder (containing 16 mg acetoxychavicol acetate), which should be divided in two doses to be consumed in the morning and the evening. The target population proposed by the applicant is "men from the normal population with a wish to father a child".

ASSESSMENT

1. Characterisation of the food/constituent

The food that is the subject of the health claim is a combination of pomegranate pomace extract and greater galangal rhizome powder.

The source of the pomegranate extract is the pomace, i.e. mashed fruit (a by-product of juice production) of pomegranate (*Punica granatum* L.), which is subjected to ethanol extraction, followed by concentration and spray drying. The extract is standardised by its content of punicalagins

(min. 30 %). The amount of total punicosides (i.e. punicalagins, punicalins, ellagic acid glycosides and ellagic acid) in the extract is at least 40 %.

The source of greater galangal powder are the rhizomes of *Alpinia galanga* (L.) Willd., which are freeze-dried and powdered (milled). The powder is standardised by its content of 1´*S*-1´-acetoxychavicol acetate (ACA; min. 4 %).

The two ingredients, pomegranate extract and greater galangal powder, may be provided as separate formulations.

An overview of the manufacturing process, batch-to-batch variability and stability data were provided for both ingredients.

The Panel considers that the food, a combination of pomegranate pomace extract (standardised by its content of punicalagins) and greater galangal rhizome powder (standardised by its content of ACA), which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is "increases the number of motile spermatozoa in semen". The target population proposed by the applicant is "men from the normal population with a wish to father a child".

The applicant was requested to define the function of the body which is the target of the claim, and to provide evidence that an improvement in this function is a beneficial physiological effect for the target population of the claim. In reply, the applicant indicated that the targeted function of the body is "the production of motile spermatozoa in man, which is essential in relation to male fertility and reproductive health". The applicant argued that "an improvement in the production of motile spermatozoa in a normal man would increase the chances of making a female partner pregnant". The applicant provided three prospective observational studies (Beltsos et al., 1996; Larsen et al., 2000; Zinaman et al., 2000) in which the percentage of motile sperm and the total number of motile sperm were significantly associated with pregnancy rates.

Spermatozoa are constituents of normal semen and are needed for fertilisation of the female ova. The total number of spermatozoa per ejaculate as well as their properties, i.e. motility, viability and morphology, are key determinants of male fertility. Reference values for sperm parameters have been established (Cooper et al., 2010; WHO, 2010). Increasing the number of motile spermatozoa in semen may contribute to the fertility of men.

The Panel considers that an increase in the number of motile spermatozoa in semen is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search in Embase, MEDLINE, Web of Science Core Collection, BIOSIS Citation Index and BIOSIS Previews. For literature related to pomegranate fruit, the following search string was used: ("Punica" or "pomegranate" or "punical*" or "ellagitannin*" or "ellagic acid" or "urolithin*) and ("sperm" or "semen" or "testosterone"). In order to retrieve literature on greater galangal, the applicant used the following search string: ("Alpinia" or "galanga*" or "acetoxychavicol acetate" or "ACA") and ("sperm" or "semen" or "testosterone"). Studies were included if they investigated any effect of 1) preparations of the rhizome of greater galangal or ACA; or 2) preparations of pomegranate fruit or ellagitannins of pomegranate fruit (including punicalagins or punicalins), or metabolites of ellagitannins (urolithins or ellagic acid), on sperm characteristics or testosterone levels. Studies not directly related to sperm quality or characteristics, or studies carried

out in diseased populations (e.g. diabetic patients) or populations outside the target population of the claim (e.g. the elderly), were excluded.

Studies investigating the effect of the food on the number of motile spermatozoa in semen

Human studies

The applicant provided one unpublished intervention study (subsequently published as Fedder, 2014) which assessed the effects of the food that is the subject of the health claim on the number of motile spermatozoa in semen (i.e. the claimed effect) *in vivo* in humans.

A double-blind, randomised, controlled, parallel trial (Fedder et al., unpublished study report, claimed as proprietary by the applicant; Fedder et al., 2014) was carried out in 70 Danish men (four participants chose to withdraw after enrolment, owing to "logistic difficulties"). The study subjects were randomised to receive pomegranate extract and greater galangal powder (n = 32, mean age 30.6 ± 7.3 years) or a placebo (microcrystalline cellulose; n = 34, mean age 28.1 ± 6.1 years) for three months. Four tablets of pomegranate extract plus four tablets of greater galangal powder were taken daily (two of each in the morning and in the evening), providing a daily dose of 1000 mg pomegranate extract and 764 mg of greater galangal powder. These amounts provided 106 mg punicalagin A, 278 mg punicalagin B, 4.7 mg punicalin, 9.6 mg ellagic acid and 16 mg ACA. In order to be eligible for inclusion in the study, participants had to be healthy adult men of at least 18 years of age with a semen quality of less than 200 million motile sperm (total motile sperm count (TMSC)) per ejaculate. Men with azoospermia were excluded from the study.

The primary outcome of the study was TMSC (determined by: ejaculate volume × spermatozoa concentration × percentage of motile spermatozoa). Assuming an expected TMSC difference of 5 million between the study groups and a standard deviation (SD) of 2.3 million, it was estimated that 18 participants per group were needed in order to achieve a statistical power of 80 % (at a significance level of 5 %). Owing to the high level of uncertainty of the assumptions in this power calculation, almost twice as many participants were enrolled. Sperm morphology was assessed as a secondary outcome. The study was approved by the Scientific Ethics Committee of Middle Jutland. The participants delivered two ejaculates at baseline (within a time span of 7–14 days and with 3–7 days of abstinence from ejaculation before each sample delivery), one ejaculate after 4–8 days of tablet intake, and two more ejaculates (within a time span of 4–10 days and with 3–7 days of abstinence from ejaculation before each sample delivery) at the end of the study (i.e. after three months). The baseline TMSC values (average of the two ejaculates ± SD) were 23.4 ± 25.1 million (95 % CI: 14.3–32.4) for the pomegranate/greater galangal-group and 19.9 ± 22.7 million (95 % CI: 12.0–27.8) for the placebo-group.

For the statistical analysis, an unequal variance t-test (i.e. Welch's t-test) was used, accounting for possible variance heterogeneity between the two groups. Data were inspected for normality using normal percentile plots (Q-Q plots). The applicant stated that there was a moderate departure from normality with a moderately right-skewed distribution. The applicant considered the t-test sufficiently robust to this moderate violation of the normality assumption. Moreover, as a confirmation analysis, all 95 % CIs and p-values were validated using the non-parametric bootstrap method. In a secondary analysis, adjustments were made in a linear regression model for age and body mass index (BMI), both dichotomised at the median. The average compliance (i.e. an intake of more than 80 % of the tablets) was 88 % in the pomegranate/greater galangal group and 91 % in the placebo group.

When the mean changes (i.e. end of the study versus baseline) in TMSC were compared between the groups, a statistically significant difference (+10.5 million; 95 % CI: 1.3–19.7, p = 0.026) was found between the pomegranate/greater galangal group (+14.5 ± 21.3 million) and the control group (+4.0 ± 15.2 million). When the analysis was adjusted for age and BMI, the difference between the groups

remained significant (+9.8 million; 95 % CI: 0.2–19.5; p = 0.047). No differences were found for the secondary outcome (i.e. sperm morphology) between the groups.

The Panel notes that this study shows an increase in the number of motile spermatozoa in semen after daily consumption of 1000 mg pomegranate pomace extract and 764 mg greater galangal rhizome powder for three months.

Animal studies

A number of animal studies, which investigated the effect of an intervention on the number of motile spermatozoa in semen, were submitted by the applicant. The studies were carried out with various preparations of greater galangal or pomegranate, or isolated compounds thereof. The Panel notes that none of these studies was conducted with a food complying with the specifications of the food for which the claim was proposed (see section 1). Therefore, the Panel considers that none of these animal studies provides evidence for an effect of the food on the number of motile spermatozoa in semen, which could support the effect observed in the one human intervention study described above.

Studies on the mechanisms by which the food could exert the claimed effect

The applicant indicated that "the exact mechanism of the combination of pomegranate extract and galangal powder in relation to sperm quality is not known". However, the applicant claims that data from some human and animal studies suggest a complementary mode of action for each of the two major constituents of the food that is the subject of the claim: greater galangal would increase blood testosterone concentrations, whereas pomegranate would exert a "direct antioxidant effect", through ellagic acid and urolithins, and an "indirect antioxidant effect", by up-regulating serum paraoxonase, which would lead to lower oxidative stress and to the protection of sperm from oxidative damage.

The applicant provided 18 human studies, 20 animal studies and two *in vitro* studies in support of a mechanism by which the two major constituents of the food could exert the claimed effect.

Two human studies (Al-Dujaili and Smail, 2012; Henning et al., 2013) and three animal studies (Amini Rad et al., 2009a, b; Bozkurt et al., 2014) were submitted as abstracts only, which did not allow a full scientific evaluation by the Panel. Therefore, no conclusions can be drawn from these studies on the mechanism by which the food could exert the claimed effect.

Human studies

The remaining 16 human studies (Aviram et al., 2000, 2004; Cerda et al., 2004; Seeram et al., 2004; Cerda et al., 2005; Mertens-Talcott et al., 2006; Seeram et al., 2006; Rock et al., 2008; Seeram et al., 2008; Hajimahmoodi et al., 2009; González-Sarrías et al., 2010; Rosenblat et al., 2010; Balbir-Gurman et al., 2011; Lynn et al., 2012; Parsaeyan et al., 2012; Basu et al., 2013) were concerned with the bioavailability of various pomegranate preparations and/or assessed outcomes on plasma total antioxidant status (e.g. by ferric reducing antioxidant potential and oxygen radical absorbance capacity assays), *ex vivo* low density lipoprotein (LDL) resistance to oxidation, serum oxidised-LDL (ox-LDL), serum anti-ox-LDL antibodies, serum malondialdehyde (MDA, determined by the thiobarbituric acid reactive substances (TBARS) assay) or serum paraoxonase activity after the consumption of various preparations of pomegranate or isolated compounds thereof. The Panel notes that none of these studies assessed whether or not the food could protect sperm from oxidative damage, or the extent to which the protection of sperm from oxidative damage could result in an increase in the number of motile spermatozoa in semen.

Animal studies

The 17 animal studies were performed in a variety of species (i.e. mice, rats, rabbits and roosters) and models (e.g. animals with chemically induced testicular and/or spermatozoal toxicity).

Two studies (Qureshi et al., 1992; Islam et al., 2000) investigated the effects of greater galangal extracts. Whereas one study (Islam et al., 2000) reported a significant increase in plasma testosterone concentrations after the consumption of a greater galangal extract compared with a placebo (number of motile spermatozoa was not assessed), the other study (Qureshi et al., 1992) reported a significant increase in the number of motile spermatozoa (testosterone concentrations were not assessed). The Panel notes that these studies do not provide evidence that greater galangal extracts induce an increase in plasma testosterone concentrations which would affect the number of motile spermatozoa in semen.

The remaining 15 animal studies (Khalil, 2004; Türk et al., 2008a, b; Atessahin et al., 2010; Ceribasi et al., 2010; Türk et al., 2010a, b; Leiva et al., 2011; Sönmez et al., 2011; Abdou et al., 2012; Ceribasi et al., 2012; Dkhil et al., 2013; Mansour et al., 2013; Shanmugam and Rama Rao, 2013; Zeweil et al., 2013) investigated the effects of various preparations of pomegranate (or isolated compounds thereof) on the activity of antioxidant enzymes (i.e. glutathione, glutathione peroxidase, superoxide dismutase, catalase) in plasma and/or testicular tissue, the concentration of MDA (determined by the TBARS assay) in plasma and/or testicular tissue, plasma testosterone concentrations, sperm motility, epididymal sperm concentrations and rates of abnormal sperm morphology. In a number of studies, an induction of the above-mentioned antioxidant enzymes and/or a decrease in the concentration of MDA (claimed to be a marker of lipid peroxidation) was observed after consumption of pomegranate preparations. The Panel notes that the induction of antioxidant enzymes is not a measure of protection against oxidative damage *per se* (EFSA NDA Panel, 2011), as the induction of such enzymes might reflect an increase in oxidative stress. The Panel notes that the studies did not investigate whether or not the induction of the antioxidant enzymes resulted in a protection of spermatozoa against oxidative damage. The Panel also notes that concentrations of MDA can only be used as supportive evidence for a protection against oxidative damage if appropriate techniques (e.g. high-performance liquid chromatography) are used for analysis (EFSA NDA Panel, 2011), which was not the case in the studies provided. Therefore, the Panel considers that these studies do not provide evidence for an effect of the pomegranate preparations used on the protection of sperm against oxidative damage or the extent to which the protection of sperm against oxidative damage might increase the number of motile spermatozoa in semen.

The Panel notes that, although the applicant did not claim a testosterone-raising effect of pomegranate, eight animal studies assessed the effects of pomegranate preparations on plasma testosterone concentrations. Six of these studies also assessed sperm motility. The Panel considers that these six studies may provide information on how changes in plasma concentrations of testosterone in response to a dietary intervention may affect the number of motile spermatozoa in semen. The results from the studies are mixed. Whereas four studies found no increase in plasma testosterone concentration but an increase in sperm motility, one study reported an increase in plasma testosterone concentration but no increase in sperm motility, and one study did not show any effects on either plasma testosterone or sperm motility. The Panel notes that these animal studies do not provide evidence that changes in plasma testosterone concentrations would consistently affect the number of motile spermatozoa in semen.

The two *in vitro* studies (Bialonska et al., 2009; Kallio et al., 2013) were performed in human immortalised cell lines and investigated the redox properties of various urolithins and urolithin derivatives. The Panel notes that the capacity of foods to scavenge free radicals *in vitro* does not provide information about their potential to decrease oxidative damage to molecules *in vivo*.

The Panel considers that the human, animal and *in vitro* studies discussed in this section do not provide evidence for a mechanism by which a combination of pomegranate pomace extract and greater galangal rhizome powder could increase the number of motile spermatozoa in semen.

Weighing the evidence

In weighing the evidence, the Panel took into account that one human study showed an increase in the number of motile spermatozoa in semen when the combination of pomegranate pomace extract and greater galangal rhizome powder was consumed for three months, that no other human studies in which these results have been replicated were provided, and that no evidence was provided for a mechanism by which the food could exert the claimed effect.

The Panel concludes that a cause and effect relationship has not been established between the consumption of the combination of pomegranate pomace extract (standardised by its content of punicalagins) and greater galangal rhizome powder (standardised by its content of ACA) and an increase in the number of motile spermatozoa in semen.

CONCLUSIONS

On the basis of the data presented, the Panel concludes that:

- The food, a combination of pomegranate pomace extract (standardised by its content of punicalagins) and greater galangal rhizome powder (standardised by its content of ACA), which is the subject of the health claim, is sufficiently characterised.
- The claimed effect proposed by the applicant is "increases the number of motile spermatozoa in semen". The target population proposed by the applicant is "men from the normal population with a wish to father a child". An increase in the number of motile spermatozoa in semen is a beneficial physiological effect.
- A cause and effect relationship has not been established between the consumption of the combination of pomegranate pomace extract (standardised by its content of punicalagins) and greater galangal rhizome powder (standardised by its content of ACA) and an increase in the number of motile spermatozoa in semen.

DOCUMENTATION PROVIDED TO EFSA

1. Health claim application on a combination of pomegranate pomace extract and greater galangal rhizome powder and an increase in the number of motile spermatozoa in semen pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (EFSA-Q-2014-00566; Claim serial No: 0424_DK). August 2014. Submitted by Nerthus ApS.

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ABBREVIATIONS

ACA	1´*S*-1´-acetoxychavicol acetate
BMI	body mass index
CI	confidence interval
LDL	low-density lipoprotein
MDA	malondialdehyde
SD	standard deviation
TBARS	thiobarbituric acid reactive substances
TMSC	total motile sperm count